(12) UK Patent Application (19) GB (11) 2 227 662 (13) A

(43) Date of A publication 08.08.1990

(21) Application No 9002016.5

(22) Date of filing 30.01.1990

(30) Priority data
(31) 305343 (32) 01.02.1989 (33) US

(71) Applicant
E.R. Squibb & Sons Inc

(Incorporated in the USA - Delaware)

Lawrenceville-Princeton Road, Princeton,
New Jersey 08543-4000, United States of America

(72) Inventors
Donald S Karanewsky
Scott Adams Biller
Eric M Gordon
William A. Scott

(74) Agent and/or Address for Service
D Young & Co
10 Staple Inn, London, WC1V 7RD, United Kingdom

(51) INT CL[5]
A61K 31/66

(52) UK CL (Edition K)
A5B BJA B28Y B281 B828 B829
U1S S2415

(56) Documents cited
None

(58) Field of search
Online databases: WPI, CHABS

(54) Lowering serum cholesterol using a HMG CoA reductase inhibitor and a squalene synthetase inhibitor

(57) A pharmaceutical combination is provided which includes an inhibitor of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, such as lovastatin, pravastatin or velostatin, and an inhibitor of the enzyme squalene synthetase. A method for reducing serum cholesterol or inhibiting formation of or treating atherosclerosis using the above combination is also provided.

GB 2 227 662 A

HMG CoA REDUCTASE INHIBITOR AND METHOD FOR LOWERING SERUM CHOLESTEROL

The present invention relates to a combination of an inhibitor of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG CoA) reductase and an inhibitor of squalene synthetase and to a method for lowering serum cholesterol and/or preventing or treating atherosclerosis by administering such combination.

There are several different classes of compounds which have serum cholesterol lowering properties. Some of these compounds are inhibitors of the enzyme HMG CoA reductase which is essential in the production of cholesterol, such as mevastatin (disclosed in U. S. Patent No. 3,983,140), lovastatin also referred to as mevinolin (disclosed in U. S. Patent No. 4,231,938), pravastatin (disclosed in U. S. Patent No. 4,346,227) and velostatin also referred to as synvinolin (disclosed in U. S. Patents Nos. 4,448,784 and 4,450,171).

Other compounds which lower serum cholesterol may do so by an entirely different mechanism than the HMG CoA reductase inhibitors. For example, serum cholesterol may be lowered through the use of bile acid sequestrants such as cholestyramine, colestipol, DEAE-Sephadex and poly(diallylmethylamine) derivatives (such as disclosed in U. S. Patents Nos. 4,759,923 and 4,027,009) or through the use of antihyperlipoproteinemics such as probucol and gemfibrozil which apparently lower serum "low density lipoproteins" (LDL) and/or converts LDL into high density lipoproteins (HDL).

U. S. Patent No. 4,759,923 mentioned above discloses that poly(diallylmethylamine) derivatives which are bile salt sequestrants may be used in conjunction with drugs which reduce serum cholesterol by mechanisms other than sequestration, such as clofibrate, nicotinic acid, probucol, neomycin, p-aminosalicylic acid or mevinolin (also referred to as lovastatin).

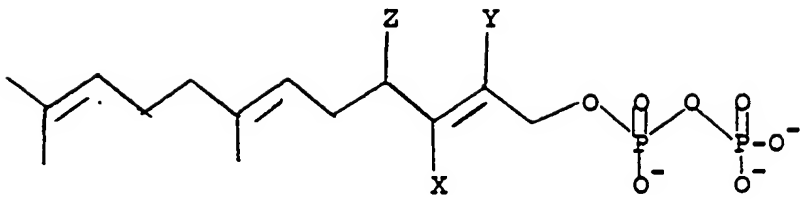
Squalene synthetase is a microsomal enzyme which catalyzes the reductive dimerization of two molecules of farnesyl pyrophosphate (FPP) in the presence of nicotinamide adenine dinucleotide phosphate (reduced form) (NADPH) to form squalene (Poulter, C. D.; Rilling, H. C., in "Biosynthesis of Isoprenoid Compounds", Vol. I, Chapter 8, pp. 413-441, J. Wiley and Sons, 1981 and references therein). This enzyme is the first committed step of the de novo cholesterol biosynthetic pathway. The selective inhibition of this step should allow the essential pathways to isopentenyl tRNA,

ubiquinone, and dolichol to proceed unimpeded. Squalene synthetase, along with HMG-CoA reductase has been shown to be down-regulated by receptor mediated LDL uptake (Faust, J. R.; Goldstein, J. L.; Brown, M. S. Proc. Nat. Acad. Sci. USA, 1979, 76, 5018-5022), lending credence to the proposal that inhibiting squalene synthetase will lead to an up-regulation of LDL receptor levels, as has been demonstrated for HMG-CoA reductase, and thus ultimately should be useful for the treatment and prevention of hypercholesterolemia and atherosclerosis.

One approach to inhibitors of squalene synthetase is to design analogs of the substrate FPP. It is clear from the literature that the pyrophosphate moiety is essential for binding to the enzyme. However, such pyrophosphates are unsuitable as components of pharmacological agents due to their chemical and enzymatic lability towards allylic C-O cleavage, as well as their susceptibility to metabolism by phosphatases.

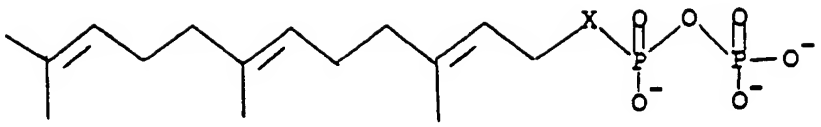
P. Ortiz de Montellano et al in J. Med. Chem., 1977, 20, 243-249 describe the preparation of a series of substituted terpenoid pyrophosphates (Table A), and have shown these to be competitive inhibitors of the squalene synthetase enzyme. These substances retain the unstable allylic pyrophosphate moiety of FPP.

Table A

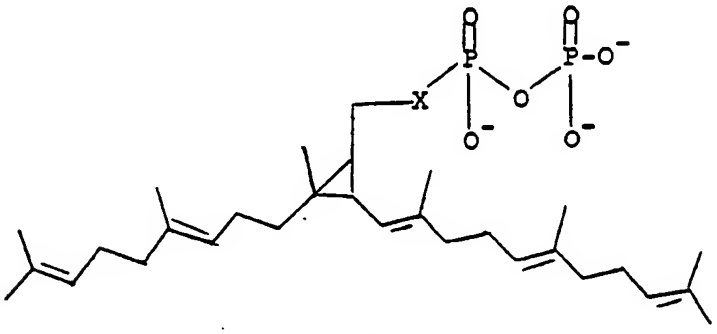


No.	X	Y	Z
1	CH_3	CH_3	H
2	H	H	H
3	C_2H_5	H	H
4	I	H	H
5	H	I	H
6	CH_3	H	SCH_3

Corey and Volante, J. Am. Chem. Soc. 1976, 98, 1291-3, have prepared FPP analog A and presqualene pyrophosphate (PSQ-PP) analog B as inhibitors of squalene biosynthesis. (Presqualene pyrophosphate is an intermediate in the conversion of FPP to squalene). These inhibitors possess methylene groups in place of the allylic oxygen moiety of FPP and PSQ-PP, but still retain the chemically and enzymatically unstable pyrophosphate linkage.

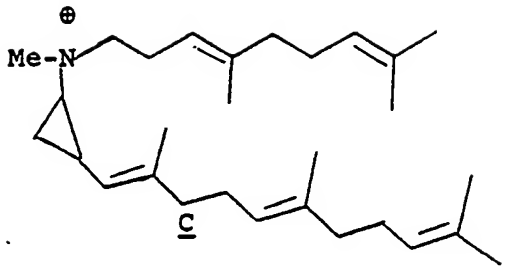


A X = CH_2
FPP X = O



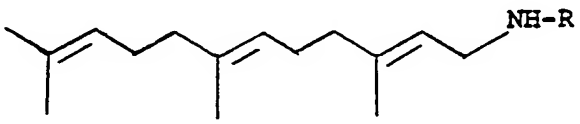
B X = CH_2
PSQ-PP X = O

Poulter and co-workers have prepared cyclopropane C (Sandifer, R. M., et al., J. Am. Chem. Soc. 1982, 104, 7376-8) which in the presence of inorganic pyrophosphate is an intermediate analog inhibitor of the enzyme squalene synthetase.



C

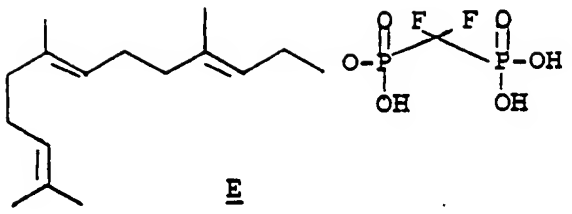
Altman and co-workers, Bertolino, A., et al., Biochim. Biophys. Acta. 1978, 530, 17-23, reported that farnesyl amine and related derivatives D inhibit squalene synthetase, but provide evidence that this inhibition is non-specific and probably related to membrane disruption.



R = H, CH_2CH_2OH, $CH_2CH_2OCH_3$

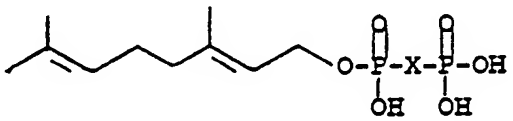
D

Poulter, C.D., et al, J. Org. Chem., 1986, 51, 4768, prepared compound E in a demonstration of a synthetic method, but did not report any biological data.



E

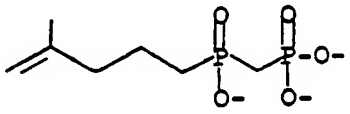
Poulter, C.D., Stremler, K.E., J.A.C.S., 1987, 109, 5542 describes the synthesis and biological evaluation of compounds having structure F. These compounds were evaluated as alternative substrates for avian liver farnesyl diphosphate and lemon peel cyclase.



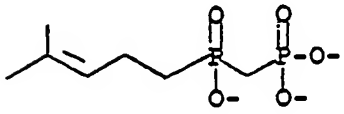
F X=CH_2, CF_2

McClard, R. W. and Poulter, C. D., et al., J.A.C.S. 1987, 109, 5544, reported that phosphinylphosphonates G and H were competitive inhibitors of the 1'-4-condensation between isopentenyl diphosphate and geranyl diphosphate catalyzed by avian liver farnesyl diphosphate synthetase. Phosphinylphosphonates G and H had Ki's of 19μM and 71μM, respectively. They also reported the speculative isolation of the farnesyl phosphinylphosphonate I, and the geranyl phosphinylphosphonate J from the enzymatic reaction of G with geranyl pyrophosphate or dimethylallyl

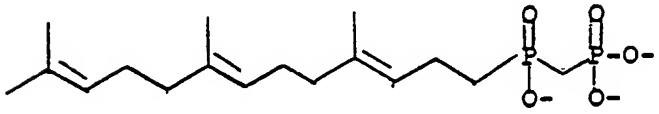
pyrophosphate, respectively. The structures of I and J were tentatively assigned based on relative TLC mobilities. They hypothesized that I could be a potential inhibitor of squalene synthetase.



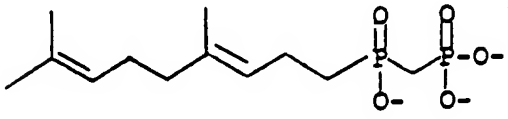
G



H

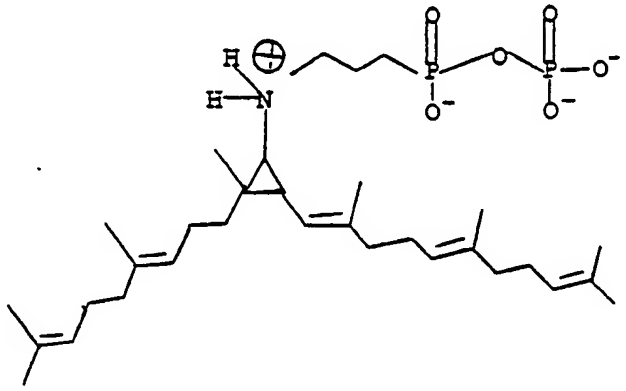


I



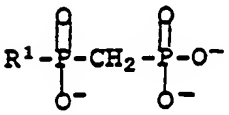
J

Capson, T.L., PhD dissertation, June 1987, Dept. of Medicinal Chemistry, the University of Utah, Abstract, Table of Contents, pp. 16, 17, 40-43, 48-51, Summary, discloses cyclopropanes of the structure discloses cyclopropanes of the structure

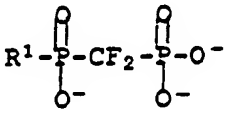


as intermediate analog inhibitors of squalene synthetase.

S. A. Biller et al., Journal of Medicinal Chemistry, 1988, Vol. 31, No. 10, pp 1869 to 1871 disclose that isoprenoid (phosphinylmethyl) phosphonates (PMPs) inhibit squalene synthetase. These phosphonates have the structures

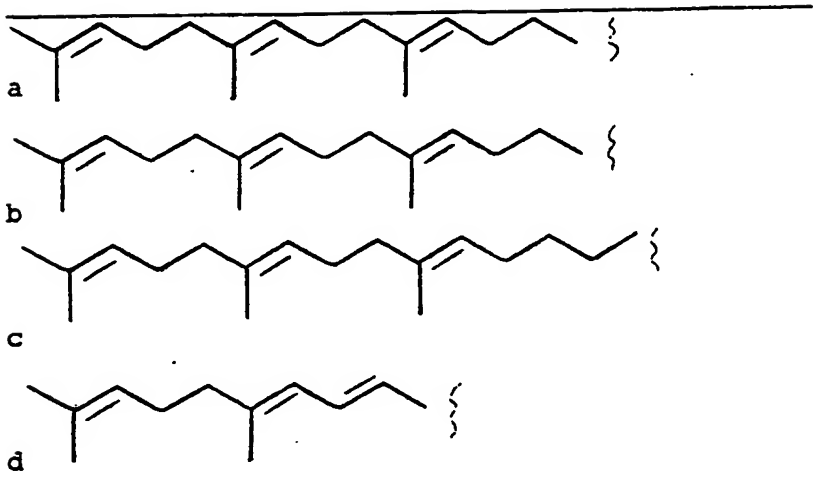


2a-d



3a,b

R^1



In accordance with the present invention, a pharmaceutical combination is provided for use in reducing serum cholesterol and in inhibiting formation of, or treating atherosclerosis, which combination is formed of an inhibitor of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase and an inhibitor of the enzyme squalene synthetase.

The HMG CoA reductase inhibitor will be employed in a weight ratio to the squalene synthetase inhibitor of within the range of from about 0.001:1 to about 1000:1 and preferably from about 0.05:1 to about 100:1.

In addition, in accordance with the present invention, a method is provided for lowering serum cholesterol or inhibiting formation of or treating atherosclerosis wherein a therapeutically effective amount of the above combination is systemically, such as orally or parenterally, administered over a prolonged period.

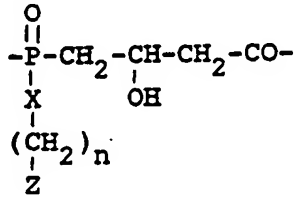
The combination of the HMG CoA reductase inhibitor and squalene synthetase inhibitor is a surprising and unique concept in inhibiting or treating elevated cholesterol and/or atherosclerosis in that it may provide additional anti-cholesterolemic effects over that which may be obtained using each of the components of the combination alone. In addition, the combination of the invention which includes compounds with different mechanisms of action, may be used to effectively treat cholesterol-related diseases of multiple etiology.

It has been found that in animal models, the HMG CoA reductase inhibitor initially inhibits cholesterol biosynthesis and also up-regulates LDL (low density lipoprotein) receptors thereby compensating for any net increase in cholesterol biosynthesis which might eventually occur. It is theorized that the squalene synthetase employed in combination with the HMG CoA reductase inhibitor, will provide another block in the cholesterol biosynthesis pathway to reduce cholesterol biosynthesis.

The HMG CoA reductase inhibitors suitable for use herein include, but are not limited to, mevastatin and related compounds as disclosed in U. S. Patent No. 3,983,140, lovastatin (mevinolin) and related compounds as disclosed in U. S. Patent No. 4,231,938, pravastatin and related compounds such as disclosed in U. S. Patent No. 4,346,227, velostatin (synvinolin) and related compounds as disclosed in U. S. Patents Nos. 4,448,784 and 4,450,171, with lovastatin, pravastatin or velostatin being preferred. Other HMG CoA

reductase inhibitors which may be employed herein include, but are not limited to, fluindostatin (Sandoz XU-62-320), pyrazole analogs of mevalonolactone derivatives as disclosed in U. S. Patent No. 4,613,610, indene analogs of mevalonolactone derivatives as disclosed in PCT application WO 86/03488, 6-[2-(substituted-pyrrol-1-yl)alkyl]-pyran-2-ones and derivatives thereof as disclosed in U. S. Patent No. 4,647,576, Searle's SC-45355 (a 3-substituted pentanedioic acid derivative) dichloroacetate, imidazole analogs of mevalonolactone as disclosed in PCT application WO 86/07054, 3-carboxy-2-hydroxy-propane-phosphonic acid derivatives as disclosed in French Patent No. 2,596,393, 2,3-di-substituted pyrrole, furan and thiophene derivatives as disclosed in European Patent Application No. 0221025, naphthyl analogs of mevalonolactone as disclosed in U. S. Patent No. 4,686,237, octahydro-naphthalenes such as disclosed in U. S. Patent No. 4,499,289, keto analogs of mevinolin (lovastatin) as disclosed in European Patent Application No. 0,142,146 A2, as well as other known HMG CoA reductase inhibitors.

In addition, compounds useful in inhibiting HMG CoA reductase suitable for use herein are disclosed in U.S. application Serial No. 182,696 filed April 18, 1988, which compounds have the moiety



wherein X is -O- or -NH-, n is 1 or 2 and Z is a hydrophobic anchor.

Examples of such compounds include (S)-4-[[[4'-fluoro-3,3',5-trimethyl[1,1'-biphenyl]-2-yl]-methoxy]methoxyphosphinyl]-3-hydroxy-butanoic acid, methyl ester or its monolithium salt,

(S)-4-[[[4'-fluoro-3,3',5-trimethyl[1,1'-biphenyl]-2-yl]methoxy]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt,

(3S)-4-[[[4'-fluoro-3,3',5-trimethyl[1,1'-biphenyl]-2-yl]methoxy]methylphosphinyl]-3-hydroxybutanoic acid, monolithium salt,

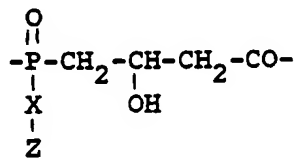
(S)-4-[[[2,4-dichloro-6-[(4-fluorophenyl)-methoxy]phenyl]methoxy]methoxyphosphinyl]-3-hydroxybutanoic acid, monolithium salt,

(3S)-4-[[[2,4-dichloro-6-[(4-fluorophenyl)-methoxy]phenyl]methoxy]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt,

(3S)-4-[[2,4-dichloro-6-[(4-fluorophenyl)-methoxy]phenyl]methoxy]methylphosphinyl]-3-hydroxybutanoic acid, or its methyl ester, and

(S)-4-[[[[4'-fluoro-3,3',5-trimethyl[1,1'-biphenyl-2-yl]methyl]amino]methoxyphosphinyl]-3-hydroxybutanoic aicd, monolithium salt.

Another class of HMG CoA reductase inhibitors suitable for use herein include compounds disclosed in U.S. application Serial No. 182,710, filed April 18, 1988, which compounds have the moiety



wherein X is $-CH_2-$ $-CH_2-CH_2-$, -CH=CH-, $-CH_2CH_2CH_2-$, -C≡C- or $-CH_2O-$, where O is linked to Z, and Z is a hydrophobic anchor.

Examples of such compounds include (S)-4-[[(E)-2-[4'-fluoro-3,3',5-trimethyl[1,1'-biphenyl]-2-yl]ethenyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt;

(S)-4-[[2-[4'-fluoro-3,3',5-trimethyl-[1,1'-biphenyl]-2-yl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, methyl ester or mono- or di-alkali metal salts thereof;

(S)-4-[[[4'-fluoro-3,3',5-trimethyl-[1,1'-biphenyl]-2-yl]ethynyl]methoxyphosphinyl]-3-hydroxybutanoic acid or the methyl ester thereof;

(5Z)-4-[[2-[4'-fluoro-3,3',5-trimethyl-[1,1'-biphenyl]-2-yl]ethenyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, methyl esters thereof;

(S)-4-[[2-[3-(4-fluorophenyl)-1-(1-methyl-ethyl)-1H-indol-2-yl]ethyl]methoxyphosphinyl]-3-hydroxybutanoic acid, methyl esters;

(S)-4-[[2-[[1,1'-biphenyl]-2-yl]ethyl]-methoxyphosphinyl-3-hydroxybutanoic acid, methyl ester;

(S)-4-[[2-[4'-fluoro-3,3',5-trimethyl-[1,1'-biphenyl]-2-yl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt;

(S)-4-[[2-[4'-fluoro-3,3',5-trimethyl-[1,1'-biphenyl]-2-yl]ethynyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt;

(SZ)-4-[[2-[4'-fluoro-3,3',5-trimethyl-[1,1'-biphenyl]-2-yl]ethenyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt;

(S)-4-[[2-[3-(4-fluorophenyl)-1-(1-methylethyl)-1H-indol-2-yl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt;

(S)-4-[[2-[(1,1'-biphenyl]-2-yl]ethyl]-hydroxyphosphinyl]-3-butanoic acid, dilithium salt;

(S)-4-(hydroxymethoxyphosphinyl)-3-[[(1,1-dimethylethyl)diphenylsilyl]oxy]butanoic acid, methyl ester, or its dicyclohexylamine (1:1) salt;

(S)-4-[[2-[1-(4-fluorophenyl)-3-(1-methylethyl)-1-indol-2-yl]ethynyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

(S)-4-[[2-[1-(4-fluorophenyl)-3-(1-methylethyl)-1H-indol-2-yl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

(E)-4-[[2-[3-(4-fluorophenyl)-1-(1-methylethyl)-1H-indol-2-yl]ethenyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

4-[[2-[4'-fluoro-3,3',5-trimethyl[1,1'-biphenyl]-2-yl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

(E)-4-[[2-[4'-fluoro-3,3',5-trimethyl[1,1'-biphenyl]-2-yl]ethenyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

(S)-4-[[[2,4-dimethyl-6-[(4-fluorophenyl)-methoxy]phenyl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

(S)-4-[[[2,4-dimethyl-6-[(4-fluorophenyl)-methoxy]phenyl]ethynyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

(S)-4-[[2-[3,5-dimethyl[1,1'-biphenyl)-2-yl]ethyl)hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

(S)-4-[[2-[4'-fluoro-3,5-dimethyl[1,1'-biphenyl]-2-yl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

(S)-4-[[2-[[1,1'-biphenyl]-2-yl]ethynyl]-hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

(S)-4-[[2-(5-(4-fluorophenyl)-3-(1-methyl-ethyl)-1-phenyl-1H-pyrazol-4-yl]ethynyl]methoxy-phosphinyl]-3-hydroxybutanoic acid, methyl ester;

(S)-4-[[2-[5-(4-fluorophenyl)-3-(1-methyl-ethyl)-1-phenyl-1H-pyrazol-4-yl]ethynyl]hydroxy-phosphinyl]-3-hydroxybutanoic acid, dilithium salt;

(E)-4-[[2-[5-(4-fluorophenyl)-3-(1-methyl-ethyl)-1-phenyl-1H-pyrazol-4-yl]ethenyl]methoxy-phosphinyl]-3-hydroxybutanoic acid, methyl ester;

(E)-4-[[2-[5-(4-fluorophenyl)-3-(1-methyl-ethyl)-1-phenyl-1H-pyrazol-4-yl]ethenyl]hydroxy-phosphinyl]-3-hydroxybutanoic acid, dilithium salt;

(S)-4-[[2-[5-(4-fluorophenyl)-3-(1-methyl-ethyl)-1-phenyl-1H-pyrazol-4-yl]ethyl]methoxy-phosphinyl]-3-hydroxybutanoic acid, methyl ester;

(S)-4-[[2-[5-(4-fluorophenyl)-3-(1-methyl-ethyl)-1-phenyl-1H-pyrazol-4-yl]ethyl]hydroxy-phosphinyl]-3-hydroxybutanoic acid, dilithium salt;

(S)-4-[[2-[3-(4-fluorophenyl)-5-(1-methylethyl)-1-phenyl-1H-pyrazol-4-yl]ethyl]methoxyphosphinyl]-3-hydroxybutanoic acid, methyl ester;

(S)-4-[[2-[3-(4-fluorophenyl)-5-(1-methylethyl)-1-phenyl-1H-pyrazol-4-yl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt;

(S)-4-[[2-[3-(4-fluorophenyl)-5-(1-methylethyl)-1-phenyl-1H-pyrazol-4-yl]ethynyl]methoxyphosphinyl]-3-hydroxybutanoic acid, methyl ester;

(S)-4-[[2-[3-(4-fluorophenyl)-5-(1-methylethyl)-1-phenyl-1H-pyrazol-4-yl]ethynyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt;

(S)-4-[[[4-(4-fluorophenyl)-1-(1-methylethyl)-3-phenyl-1H-pyrazol-5-yl]ethynyl]methoxyphosphinyl]-3-hydroxybutanoic acid, methyl ester;

(S)-4-[[[4-(4-fluorophenyl)-1-(1-methylethyl)-3-phenyl-1H-pyrazol-5-yl]ethynyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt;

(S)-4-[[2-[4-(4-fluorophenyl)-1-(1-methylethyl)-3-phenyl-1H-pyrazol-5-yl]ethyl]methoxyphosphinyl]-3-hydroxybutanoic acid, methyl ester;

(S)-4-[[2-[4-(4-fluorophenyl)-1-(1-methylethyl)-3-phenyl-1H-pyrazol-5-yl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt;

(S)-4-[[[1-(4-fluorophenyl)-4-(1-methylethyl)-2-phenyl-1H-imidazole-5-yl]ethynyl]methoxyphosphinyl]-3-hydroxybutanoic acid, methyl ester;

(S)-4-[[[1-(4-fluorophenyl)-4-(1-methylethyl)-2-phenyl-1H-imidazol-5-yl]ethynyl]methoxyphosphinyl]-3-hydroxybutanoic acid, methyl ester;

(S)-4-[[2-[1-(4-fluorophenyl)-4-(1-methylethyl)-2-phenyl-1H-imidazol-5-yl]ethyl]methoxyphosphinyl]-3-hydroxybutanoic acid, methyl ester;

(S)-4-[[2-[1-(4-fluorophenyl)-4-(1-methyl-ethyl)-2-phenyl-1H-imidazol-5-yl]ethyl]hydroxy-phosphinyl]-3-hydroxybutanoic acid, dilithium salt;

(S)-4-[[[2-(cyclohexylmethyl)-4,6-dimethyl-phenyl]ethynyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

4-[[2-[2-(cyclohexylmethyl)-4,6-dimethyl-phenyl]ethenyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

(S)-4-[[2-[2-(cyclohexylmethyl)-4,6-dimethyl-phenyl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

4-[[[[4'-fluoro-3,3',5-trimethyl[1,1'-biphenyl]-2-yl]oxy]methyl]hydroxyphosphinyl]-3-hydroxybutanoic acid or its dilithium salt or methyl ester thereof;

4-[[[4'-fluoro-3,3',5-trimethyl[1,1'-biphenyl]-2-yl]methyl]hydroxyphosphinyl]-3-hydroxy-butanoic acid or its dilithium salt or methyl ester thereof;

(S)-4-[[[1-(4-fluorophenyl)-3-methyl-2-naphthalenyl]ethynyl]hydroxyphosphinyl]-3-hydroxy-butanoic acid or its dilithium salt or methyl ester thereof;

(E)-4-[[2-[1-(4-fluorophenyl)-3-methyl-2-naphthalenyl]ethenyl]hydroxyphosphinyl]-3-hydroxy-butanoic acid or its dilithium salt or methyl ester thereof;

(S)-4-[[2-[1-(4-fluorophenyl)-3-methyl-2-naphthalenyl]ethyl]hydroxyphosphinyl]-3-hydroxy-butanoic acid or its dilithium salt or methyl ester thereof;

4-[[3-[4'-fluoro-3,3',5-trimethyl[1,1'-biphenyl]-2-yl]propyl]methoxyphosphinyl]-3-hydroxybutanoic acid, methyl ester;

4-[[3-[4'-fluoro-3,3',5-trimethyl[1,1'-biphenyl]-2-yl]propyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt;

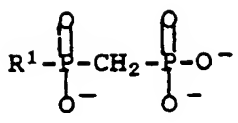
[1S-[1<a(R*),2<a,4a<b,8<b,8a<a]]-4-[[2-[8-(2,2-dimethyl-1-oxobutoxy)decahydro-2-methyl-1-naphthalenyl]ethyl]methoxyphosphinyl]-3-hydroxybutanoic acid, methyl ester;

[1S-[1<a(R*),2<a,4a<b,8<b,8a<a]]-4-[[2-[8-(2,2-dimethyl-1-oxobutoxy)decahydro-2-methyl-1-naphthalenyl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt;

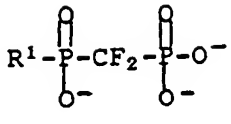
(S)-4-[[[3'-(4-fluorophenyl)spiro]cyclopentane-1,1'-[1H]indene]-2-yl]ethynyl]methoxyphosphinyl]-3-hydroxybutanoic acid, methyl ester; and

(S)-4-[[[3'-(4-fluorophenyl)spiro]cyclopentane-1,1'-[1H]indene]-2-yl]ethynyl]hydroxyphosphinyl]-3-hydroxybutanoic acid, dilithium salt.

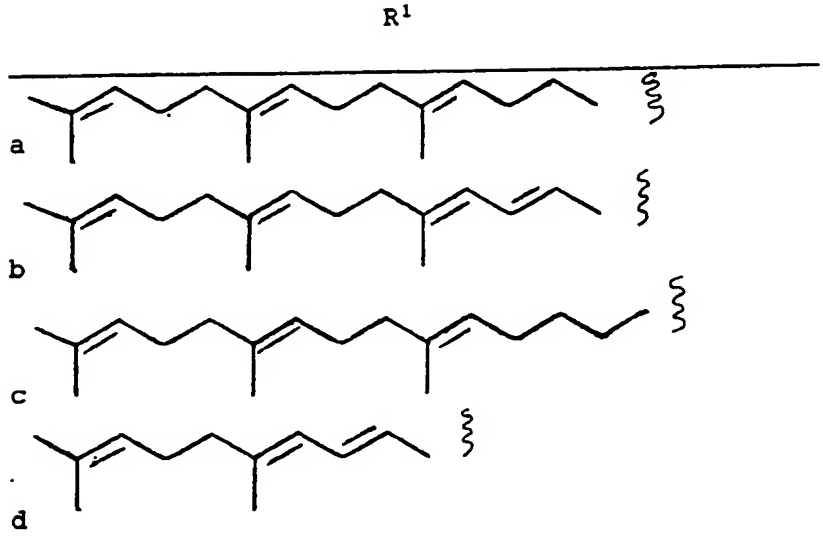
The squalene synthetase inhibitors suitable for use herein include, but are not limited to, those disclosed by Biller et al., supra, including isoprenoid (phosphinylmethyl)phosphonates such as those of the formula



I



II



including the triacids thereof, triesters thereof and tripotassium and trisodium salts thereof as well as other squalene synthetase inhibitors disclosed in European Patent Application EP-A-324421 published 19th July 1989.

In addition, other squalene synthetase inhibitors suitable for use herein include the terpenoid pyrophosphates disclosed by P. Ortiz de Montellano et al., J. Med. Chem.; 1977, 20, 243-249, the farnesyl diphosphate analog A and presqualene pyrophosphate (PSQ-PP) analogs as

disclosed by Corey and Volante, J. Am. Chem. Soc. 1976, 98, 1291-1293, phosphinylphosphonates reported by McClard, R. W. et al., J.A.C.S., 1987, 109, 5544 and cyclopropanes reported by Capson, T.L., PhD dissertation, June, 1987, Dept. Med. Chem. U. of Utah, Abstract, Table of Contents, pp. 16, 17, 40-43, 48-51, Summary.

The disclosure of the above-mentioned patents and patent applications are incorporated herein by reference.

Preferred are combinations of lovastatin, pravastatin or velostatin with a squalene synthetase inhibitor such as disclosed by Biller et al., supra.

In carrying out the method of the present invention, the combination of the invention may be administered to mammalian species, such as monkeys, dogs, cats, rats, humans, etc. and as such may be incorporated in a conventional systemic dosage form, such as a tablet, capsule, elixir or injectable. The above dosage forms will also include the necessary carrier material, excipient, lubricant, buffer, antibacterial, bulking agent (such as mannitol), anti-oxidants (ascorbic acid of sodium bisulfite) or the like. Oral dosage forms are preferred, although parenteral forms are quite satisfactory as well.

The dose administered must be carefully adjusted according to age, weight and condition of the patient, as well as the route of administration, dosage form and regimen and the desired result.

Thus, for oral administration, a satisfactory result may be obtained employing the HMG CoA reductase inhibitor in dosages employed, for example, for lovastatin as indicated in the Physician's Desk Reference, such as in an amount within the range of from about 1 to 2000 mg, and preferably from about 4 to about 200 mg in combination with the squalene synthetase inhibitor in dosages in an amount within the range of from about 10 mg to about 2000 mg and preferably from about 25 mg to about 200 mg with the HMG CoA reductase inhibitor and squalene synthetase inhibitor being employed together in the same oral dosage form or in separate oral dosage forms taken at the same time.

A preferred oral dosage form, such as tablets or capsules, will contain the HMG CoA reductase inhibitor in an amount of from about 0.1 to about 100 mg, preferably from about 5 to about 80 mg, and more preferably from about 10 to about 40 mg, and the squalene synthetase inhibitor in an amount of from about 10 to about 500 mg, preferably from about 25 to about 200 mg.

The composition described above may be administered in the dosage forms as described above in single or divided doses of one to four times daily. It may be advisable to start a patient on a low dose combination and work up gradually to a high dose combination.

Tablets of various sizes can be prepared, e.g., of about 2 to 2000 mg in total weight, containing one or both of the active substances in the ranges described above, with the remainder

being a physiologically acceptable carrier of other materials according to accepted pharmaceutical practice. These tablets can, of course, be scored to provide for fractional doses. Gelatin capsules can be similarly formulated.

Liquid formulations can also be prepared by dissolving or suspending one or the combination of active substances in a conventional liquid vehicle acceptable for pharmaceutical administration so as to provide the desired dosage in one to four teaspoonsful.

Such dosage forms can be administered to the patient on a regimen of one to four doses per day.

According to another modification, in order to more finely regulate the dosage schedule, the active substances may be administered separately in individual dosage units at the same time or carefully coordinated times. Since blood levels are built up and maintained by a regulated schedule of administration, the same result is achieved by the simultaneous presence of the two substances. The respective substances can be individually formulated in separate unit dosage forms in a manner similar to that described above.

Fixed combinations of HMG CoA reductase inhibitor and squalene synthetase inhibitors are more convenient and are preferred, especially in tablet or capsule form for oral administration.

In formulating the compositions, the active substances, in the amounts described above, are compounded according to accepted pharmaceutical practice with a physiologically acceptable vehicle, carrier, excipient, binder, preservative,

stabilizer, flavor, etc., in the particular type of unit dosage form.

Illustrative of the adjuvants which may be incorporated in tablets are the following: a binder such as gum tragacanth, acacia, corn starch or gelatin; an excipient such as dicalcium phosphate or cellulose; a disintegrating agent such as corn starch, potato starch, alginic acid or the like; a lubricant such as stearic acid or magnesium stearate; a sweetening agent such as sucrose, aspartame, lactose or saccharin; a flavoring agent such as orange, peppermint, oil of wintergreen or cherry. When the dosage unit form is a capsule, it may contain in addition to materials of the above type a liquid carrier such as a fatty oil. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets or capsules may be coated with shellac, sugar or both. A syrup of elixir may contain the active compound, water, alcohol or the like as the carrier, glycerol as solubilizer, sucrose as sweetening agent, methyl and propyl parabens as preservatives, a dye and a flavoring such as cherry or orange.

Some of the active substances described: above form commonly known, pharmaceutically acceptable salts such as alkali metal and other common basic salts or acid addition salts, etc. References to the base substances are therefore intended to include those common salts known to be substantially equivalent to the parent compound.

The formulations as described above will be administered for a prolonged period, that is, for as long as the potential for elevated serum cholesterol and atherosclerosis remains or the symptoms continue. Sustained release forms of such formulations which may provide such amounts biweekly, weekly, monthly and the like may also be employed. A dosing period of at least one to two weeks are required to achieve minimal benefit.

The following Examples represent preferred embodiments of the present invention. All temperatures are expressed in degrees Centigrade unless otherwise indicated and all mesh sizes are U.S. Standard ASTME.

Example 1

A pravastatin formulation in the form of tablets having the following composition was prepared as described below.

Ingredient	Parts by Weight
Pravastatin	7
Lactose	67
Microcrystalline cellulose	20
Croscarmellose sodium	2
Magnesium stearate	1
Magnesium oxide	3

Pravastatin, magnesium oxide and a fraction (30%) of the lactose were mixed together for 2 to 10 minutes employing a suitable mixer. The resulting mixture was passed through a #12 to #40 mesh size screen. Microcrystalline cellulose, croscarmellose sodium and the remaining lactose were added and the mixture was mixed for 2 to 10 minutes. Thereafter, magnesium stearate was added and mixing was continued for 1 to 3 minutes.

The resulting homogeneous mixture was then compressed into tablets each containing 5 mg, 10 mg, 20 or 40 mg pravastatin.

Tablets each containing the following ingredients:

Ingredient	Weight (mg)
(E,E)-[difluoro[hydroxy(4,8,12-trimethyl-3,7,11-tridecatrienyl)-phosphinyl]methyl]phosphonic acid tripotassium salt (squalene synthetase inhibitor prepared as described by Biller et al. supra)	100 mg
Avicel	112.5 mg
Lactose	113 mg
Cornstarch	17.5 mg
Stearic Acid	7 mg
	350 mg

are prepared from sufficient bulk quantities by slugging the squalene synthetase inhibitor Avicel, and a portion of the stearic acid. The slugs are ground and passed through a #2 screen and then mixed with the lactose, cornstarch, and the remainder of stearic acid. The mixture is compressed into 350 mg capsule shaped tablets in a tablet press. The tablets are scored for dividing in half.

The pravastatin tablets and squalene synthetase inhibitor tablets may be administered as a combination in accordance with the teachings of the present invention to lower serum cholesterol and/or treat atherosclerosis. In addition, the pravastatin and squalene synthetase inhibitor tablets may be ground up into powders and used together in a single capsule.

Example 2

A pravastatin formulation in the form of tablets, each containing 5 mg, 10 mg, 20 mg or 40 mg pravastatin, having the following composition was prepared as described in Example 1, except that color was incorporated into the powder mixture containing pravastatin, magnesium oxide and a fraction of the lactose.

Ingredient	Parts by Weight
Pravastatin	7
Lactose	67
Microcrystalline cellulose	20
Croscarmellose sodium	2
Magnesium stearate	1
Magnesium oxide	3
FD&C Red #3 Lake	0.2

The pravastatin tablet and squalene synthetase inhibitor tablet (described in Example 1) may be administered as a combination or the pravastatin tablet and squalene synthetase inhibitor tablet may be ground into powders and used in a single capsule to lower serum cholesterol and/or treat atherosclerosis in accordance with the teachings of the present invention.

Examples 3 and 4

Lovastatin tablets are prepared employing conventional pharmaceutical techniques containing 20 mg lovastatin, cellulose, color, lactose, magnesium stearate and starch and butylated

hydroxyanisole as a preservative as described in the 1988 PDR.

The lovastatin tablets may be employed in combination with the squalene synthetase inhibitor tablets (described in Examples 1 and 2) in separate or combined dosage forms to treat elevated serum cholesterol or atherosclerosis in accordance with the present invention.

Examples 5 to 7

A formulation in the form of tablets having the following composition is prepared as described in Example 1.

Ingredient	Weight (mg)
(E,E,E)-[difluoro[hydroxy(4,8,12-trimethyl-1,3,7,11-tridecatetraenyl)phosphinyl]methyl]-phosphonic acid tripotassium salt (squalene synthetase inhibitor prepared as described by Biller et al. supra)	100 mg
Cornstarch	50 mg
Gelatin	7.5 mg
Avicel (microcrystalline cellulose)	25 mg
Magnesium stearate	2.5 mg
	185 mg

Pravastatin tablets, or lovastatin tablets described in Examples 1 and 3, respectively, or velostatin tablets may be employed in combination with the above squalene synthetase inhibitor

tablets. The pravastatin or lovastatin and squalene synthetase inhibitor may be employed in separate dosage forms or combined in a single capsule form to lower elevated serum cholesterol or treat atherosclerosis in accordance with the present invention.

It will be appreciated that any of the HMG CoA reductase inhibitors disclosed herein may be employed in combination with any of the squalene synthetase inhibitors disclosed by Biller et al. supra and in EP-A-324421.

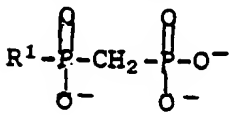
CLAIMS

1. A pharmaceutical combination comprising an inhibitor of the enzyme 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG CoA) reductase and an inhibitor of the enzyme squalene synthetase.

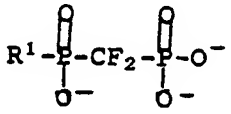
2. The combination as defined in Claim 1 wherein said inhibitor of the enzyme HMG CoA reductase is mevastatin, lovastatin, pravastatin or velostatin.

3. The combination as defined in Claim 1 wherein said inhibitor of the enzyme HMG CoA reductase is a pyrazole analog of a mevalono-lactone, an indene analog of mevalonolactone, a 3-carboxy-2-hydroxy-propane-phosphonic acid derivative, a 6-[2-(substituted-pyrrol-1-yl)-alkyl]pyran-2-one, an imidazole analog of mevalonolactone, or a heterocyclic analog of mevalonolactone, a naphthyl analog of mevalono-lactone, an octahydro-naphthalene, fluindostatin, a keto analog of lovastatin or a 2,3-di-substituted pyrrole, furan or thiophene.

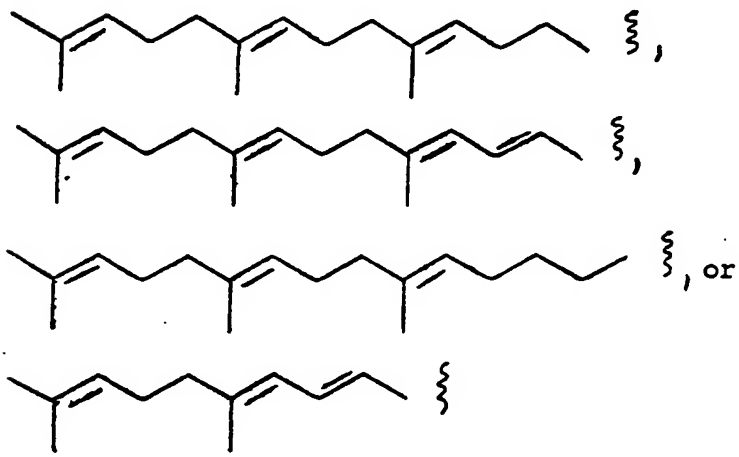
4. The combination as defined in Claim 1 wherein the inhibitor of the enzyme squalene synthetase has the formula



or



wherein R^1 is



5. The combination as defined in Claim 1 wherein the inhibitor of the enzyme HMG CoA reductase is present in a weight ratio to said inhibitor of the enzyme squalene synthetase of within the range of from about 0.001:1 to about 1000:1.

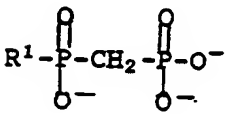
6. The combination as defined in Claim 4 wherein the inhibitor of the enzyme HMG CoA reductase is lovastatin, pravastatin or velostatin.

7. The combination as defined in Claim 4 wherein the inhibitor of the enzyme HMG CoA reductase is lovastatin, pravastatin or velostatin.

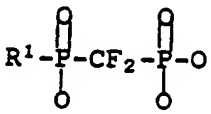
8. A method for lowering serum cholesterol or inhibiting formation of or treating atherosclerosis, which comprises administering to a patient in need of such treatment a therapeutically effective amount of a pharmaceutical combination comprising an inhibitor of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase and an inhibitor of the enzyme squalene synthetase.

9. The method as defined in Claim 8 wherein said inhibitor of enzyme HMG CoA reductase is mevastatin, lovastatin, pravastatin or velostatin.

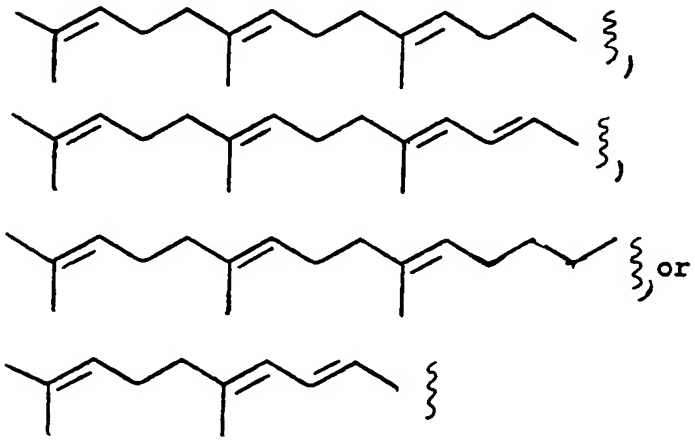
10. The method as defined in Claim 8 wherein the inhibitor of the enzyme squalene synthetase has the formula



or



wherein R^1 is



11. The method as defined in Claim 10 wherein the inhibitor of the enzyme HMG CoA reductase is lovastatin, pravastatin or velostatin.

12. A hypocholesterolemic or hypolipemic composition comprising a combination as defined in Claim 1 and a pharmaceutically acceptable carrier therefor.

Published 1990 at The Patent Office, State House, 66 71 High Holborn, London WC1R 4TP. Further copies may be obtained from: The Patent Office, Sales Branch, St Mary Cray, Orpington, Kent BR5 3RD. Printed by Multiplex techniques ltd, St Mary Cray, Kent, Con. 1 87